UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE

SO

REPLY TO: 1630 Written Information

September 19, 1975

SUBJECT: Study Plan FS-SO-2203-1.36
Cooperative Agreement #19-209

TO: T. A. Harrington, AD-WG

Enclosed is the study plan "PRODUCTION OF SOUTHERN PINE BEETLES FREE OF MICROBES IN THE GUT FOR USE IN STUDIES ON SOURCE OF PHEROMONES", a blue line summary, and approval page, and the biometrics review.

STANLEY J. BARRAS
Project Leader
RWU 2203

Enclosures

4500
FS-SO-2203-1.36
Problem 2

September 1975
Study Plan Summary
James E. Marler and
Stanley J. Barras - Alexandria, La.

PRODUCTION OF SOUTHERN PINE BEETLES FREE OF MICROBES IN THE GUT FOR USE IN STUDIES ON SOURCE OF PHEROMONES

(Cooperative Agreement #19-209)

Studies on pheromone production and scholidis and the southern pine beetle in particular have not shown the source of compounds. There are indications that microorganisms can play a significant role in converting tree-terpenes such as α-pinene into cis- and trans-verbenol, verbenones and other compounds. Recent studies have indicated that *Bacillus cereus* from the SPB is capable of pheromone production and that a mycangial fungus had similar capabilities. Barras and Marler showed that *B. cereus* and other bacteria are common inhabitants of gut and galleries of the SPB. Because the SPB has not been shown to produce pheromones and cells associated with the gut tissue the possibility exists that the insect *per se* does not produce most or all of the behavioral chemical claimed to originate with the insect. This study will have a two-fold purpose. 1) To produce SPB free of internal and external microbes and 2) cooperate with Dr. J. M. Brand in the following aspects: supply Dr. Brand with microorganism free pine beetles, isolate and identify microbes (mainly bacteria and yeasts) required by Dr. Brand from the gut and/or galleries of SPB for use in study on influence of microorganisms on pheromonal regimes of the SPB.

UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

4500
FS-SO-2203-1.36

STUDY PLAN

PRODUCTION OF SOUTHERN PINE BEETLES FREE OF MICROBES IN THE GUT FOR USE IN STUDIES ON SOURCE OF PHEROMONES

(Cooperative Agreement #19-209)

James E. Marler
and
Stanley J. Barras

RWU 2203

Forest Insect Research Project
Alexandria, Louisiana

Southern Forest Experiment Station

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Problem 2

STUDY PLAN

PRODUCTION OF SOUTHERN PINE BEETLES FREE OF MICROBES

IN THE GUT FOR USE IN STUDIES ON SOURCE OF PHEROMONES

A cooperative agreement with Louisiana State University
at Alexandria and Southern Forest Experiment Station

Prepared by:

James E. Marler 8/8/75

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Stanley J. Barras 8/8/75

Dr. Stanley J. Barras (Date)
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Approved by:

9/9/75

Dr. Stanley J. Barras (Date)
Project Leader, Forest Insect Research
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Alexandria, Louisiana 71301

4500
FS-SO-2203-1.36
Problem 2

STUDY PLAN

PRODUCTION OF SOUTHERN PINE BEETLES FREE OF MICROBES IN THE GUT FOR USE IN STUDIES ON SOURCE OF PHEROMONES

A cooperative agreement with Louisiana State University at Alexandria and Southern Forest Experiment Station
(Cooperative Agreement #19-209)

INTRODUCTION

The association of microbes with various insects is well documented (11, 15). Many have reviewed the microbes with an eye toward natural control of insect populations (14) but seldom is the relationship of the microbe and its host insect delved into very deeply. Many bacteria, yeast, and fungi are associated with the Southern Pine Beetle, *Dendroctonus frontalis* (4, 5, 6, 12) and their role in the insect's life cycle is currently being investigated. One aspect of this relationship is the role of associated microbes in the production of pheromones eliciting behavioral response in the southern pine beetle (SPB).

Studies on pheromone production in scolytids and SPB in particular have not shown the source of compounds. There are indications that micro-organisms can play a significant role in converting tree terpenes such as α-pinene into cis and trans-verbenol, verbenone, and other compounds (7, 9).

Recent studies (8, 8b) have indicated that *Bacillus cereus* from the SPB is capable of pheromone production and that a mycangial fungus (6) had similar capabilities (9). Barras and Marler (4, 5) showed that *B. cereus* and other bacteria are common inhabitants of the gut and galleries of the SPB. Because the SPB has not been shown to produce pheromones in cells associated with the gut tissue the possibility exists that the insect *per se*, does not produce most or all of the behaviorial chemicals claimed to originate with the insect.

OBJECTIVES OF STUDY

This study will have a two fold objective: 1) to produce SPB free of internal and external microbes; 2) cooperate with Dr. J. M. Brand in the following aspects: supply JMB with microbe-free pine beetles, isolate and identify microbes (mainly bacteria and yeasts) required by JMB from the gut and/or galleries of SPB for use in study on influence of microorganisms on pheromonal regime of SPB (8).

Production of microbe free SPB will allow us to determine the actual role of gut microbes in the pheromone production process. If the microbes are the primary source of pheromones then microbe-free SPB should fail to convert α-pinene to cis/trans verbenol, verbenone, or even produce other pheromonally active compounds.

METHODS

Pupae will be gathered from field collected bolts and surfaced sterilized in the manner described by Barras (1, 2). After molting into callow adults, the surface sterilized beetles will be placed on an aritificial diet based on previously developed diets (3) but containing antimicrobial agents to eliminate the intestinal flora. The agents recommended by Singh and House (13) and Thiele (16) will be pre-screened for their effectiveness in surpressing the growth of typical SPB host microbes. The following microorganisms will be used to determine the effective level of each agent used: Bacteria-*Bacillus cereus*, *Micrococcus luteus*, *Serretia marcescens*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*; Yeast-Candida sp.; Hyphal fungi-*Ceratocystis minor*, *Aspergillus achrecous*, *Penicillum sp*, and mycangial fungi #122 and #133.

The SPB will be placed in plates containing the test diet and allowed to borrow for 3 days. The beetles will be analyzed for microbial content in standard microbial assay media (Malt extract agar for yeast/fungi and trypic soytone agar/broth for bacteria).

Microbe-free beetles will be shipped in sterile containers to Brand for analysis of phermonal activity.

Isolation and identification of gut and gallery microbes will be done by standard microbial methods, such as used in previous SPB studies (4, 5).

FACILITIES

The equipment and facilities required for this project are available at Louisiana State University at Alexandria per agreement # _____. All supervision will be provided by Dr. James Marler.

PRESENTATION OF RESULTS

All reports will be prepared and presented to the Forest Service as required in the cooperative agreement. LSU-A and the University of Iowa will cooperate in sharing results to coordinate both final reports.

BIBLIOGRAPHY

1. Barras, S. J.

   1972. Improved White's solution for surface sterilization of pupae of *Dendroctonus frontalis*, J. Eco. Emto. 65:1504.

2. _____

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3. _____

   1975. Development of an onipositional diet for the Southern Pine Beetle. Final Report FS-50-2203-1.19, USDA FS Sou. For. Exp. Sta.

4. _____ and Marler, J. E.

   1974. Identification of bacterial flora in galleries of the SPB. *Dendroctonus frontalis* (Zimm.) Study Plan FS-50-2203-1.29, USDA, F.S. Sou. For. Exp. Sta.

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13. Singh, Pritam, and House, H. L.

1970. Antimicrobials: Safe levels in a synthetic diet of an insect, *Agria affinis*. J. Insect. Physiol. 16: 1769-82.

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Personal communications - Schering Corp. Sales Representative.

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BIOMETRICS REVIEW ROUTING

ROUTING

~~1) Biometrics~~
~~2) RWU 2203~~ SJB 9/9/75
3) AD-WG

RESEARCH WORK UNIT

[X] STUDY PLAN [ ] MANUSCRIPT [ ] RESPONSE TO BIOMETRICS REVIEW [ ] _____ (OTHER)

FS-SO-2203-1.36 "PRODUCTION OF SOUTHERN PINE BEETLES FREE OF MICROBES IN THE GUT FOR USE IN STUDIES ON SOURCE OF PHEROMONES"

(STUDY NUMBER) (TITLE)

Stanley J. Barras/sem 8/11/75

(AUTHOR SIGNATURE) (DATE)

REMARKS:

SUBMITTED: John Moser 8/11/75

(PROJECT LEADER SIGNATURE) (DATE)

Acting Project Leader

AD

[ ] FORWARDED FOR BIOMETRICS REVIEW [ ] BIOMETRICS REVIEW WAIVED

(ASSISTANT DIRECTOR SIGNATURE) (DATE)

BIOMETRICS SERVICES

[X] NO COMMENTS [ ] COMMENTS BELOW [ ] COMMENTS ATTACHED

[ ] COMMENTS MADE BY PHONE

IN 8/15/75

T.R. Dell 5 SEP 75

(BIOMETRICIAN SIGNATURE) (DATE)

OUT 5 SEP 75

COMMENTS:

INSTRUCTIONS

1. Project Leader must submit this form with each study plan. Also use this form when manuscripts are submitted for review.
2. Insert desired "Routing" in space at top of form.
3. Be sure to insert proper identification in the "Research Work Unit" block.
4. Completed copies of this form will be filed in the study file in the Assistant Director's and Project Leader's files.

ORIGINAL – RESEARCH WORK UNIT FILE

S T U D Y P L A N

Determination of the source of pheromonal activity in the gut of the Southern Pine Beetle.

A cooperative agreement with Louisiana State University at Alexandria and Southern Forest Experiment Station.

by

Dr. James E. Marler
Div. of Sciences
L.S.U. at Alexandria
Alexandria, LA 71301

Dr. Stanley J. Barras
Southern Forest Exp. Station
Forest Insect Research
Pineville, LA 71360

INTRODUCTION

The association of microbes with various insects is well documented (9, 13). Many have reviewed the microbes found with an eye toward natural control of insect populations (12) but seldom is the relationship of the microbe and its host insect delved into very deeply. Many bacteria, yeast, and fungi are associated with the Southern Pine Beetle, Dendroctonus frontalis (4,5,6,10) and their role in the insect's life cycle is currently being investigated. One aspect of this relationship is the role of pheromones and microbes associated with Southern Pine Beetle.

Studies into the source of pheromone production indicate that micro-organisms can play a significant role in converting α- pinene into cis and trans-verbenol, verbenone, and other compound (7). Still undetermined is the role of the beetle in this pheromone production. Studies by Barras and Brand (8) indicated that certain bacteria isolated from the frass of Southern Pine Beetle were capable of pheromone production. Barras and Marler (4, 5) showed that these bacteria are common inhabitants of the gut and galleries of the Southern Pine Beetle. Therefore, the question arises, does the pine beetle have a significant role in pheromone production, and if so, what part of the gut is involved?

OBJECTIVES OF STUDY

This study will have two fold objective: 1) to produce SPB free of internal or external microbes; 2) cooperate with Dr. J. M. Brand in the following aspects: supply JMB with microbe-free pine beetles, isolate and identify microbes required by JMB from the gut and/or galleries of SPB.

Production of microbe free SPB will allow us to determine the actual role of microbes in the phermone production processes. If the microbes are the primary source of pheromones than microbe-free SPB should fail to convert α-pinene to cis/trans verbenol, verbenone, or even produce other pheromonally active compounds.

METHODS

The pupae will be gathered from field collected bolts and surfaced sterilized in the manner described by Barras (1, 2). After molting into callow adults, the surface sterilized beetles will be placed on an artificial diet. This diet will be based on previously developed diets (3). Various combinations of antimicrobial agents will be screened for their effectiveness in surpressing the growth of typical SPB host microbes. The following microorganisms will be used to determine the effective-level of each agent used: Bacteria-*Bacillus cereus*, *Micrococcus luteus*, *Serretia marcescens*, *Enterobactes aerogenes*, and *Pseudomonas aeruginosa*; Yeast- *Candida* sp.; Hyphal fungi - *Ceratocystis minor*, *Aspergillus ochrecous*, *Penicillum sp* , #122, and #133. The antimicrobial agents used will be taken from those recommended by Singh and House (11) and Thiele (14).

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